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(54) TEST STRIPS

(71) We, BOEHRINGER MANNHEIM G.M.B.H., of 112-132 Sandhofer Strasse, Mannheim-Waldhof, Germany, a Body Corporate organised under the laws of Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with test strips for the detection of substances in solution in liquids, especially in body fluids.

Reagent papers have been in use for a long time, pH indicator papers being the most widely used but other reagent papers are also employed: for example, curcuma paper is used for the detection of acids, potassium iodide-starch paper for the detection of oxidation agents and lead acetate paper for the detection of sulphides. Recently, test papers have also achieved considerable importance in the field of clinical chemistry. They permit the rapid, easy and semi-quantitative determination of pathological components, such as glucose, protein and the like, in body fluids, such as urine, serum and the like. Furthermore, since test papers of this type are used to an ever greater extent by lay persons, it has proved to be necessary to bring the test papers, which merely consist of impregnated filter paper, into a form which is safer to use and with which disturbances of the detection reaction can be avoided.

One of the most common sources of disturbances are faulty reactions of the test papers, brought about by contact with the hands or by external influences, for example moisture and autoxidation. In order to avoid touching the test paper, it is now usual to affix the test paper, in the form of a small rectangle, on to a strip of plastics material which not only serves as a handle but also simultaneously results in the saving of reagents, which are often expensive.

For the protection of sensitive reagents against the action of atmospheric moisture, oxygen and the like, the test zones have also been sealed betwen synthetic resin foils in such a manner that the liquid to be investigated can only reach the test zone *via* the uncovered edges of the test zone (cf. published German Patent Specification No. 1,546,307). This method admittedly provides a good protection for the test paper but, nevertheless, it is necessary to put up with some serious disadvantages, the avoidance of which has hitherto not been successful, in spite of intensive efforts. Admittedly, the test papers in the test strips according to published German Patent Specification No. 1,546,307 are, surprisingly, also rapidly and completely wetted with liquid, even when dipped into the test liquid for only a short time. Nevertheless, it is possible for air bubbles to be formed between the paper and the synthetic resin foil, which can considerably complicate the evaluation of the coloration of the test zone. Furthermore, it can sometimes happen that, in the case of lateral penetration of liquid, disturbing chromatographic effects can occur, resulting in a non-uniform coloration of the test zone, which is difficult to evaluate.

In order to overcome these deficiencies at least partially, the protective synthetic resin foils have been provided with holes (cf. published German Patent Specification No. 1,546,307). Such tests strips appear to the observer to be coarsely graticulated since, for technical reasons, per test zone there can only be punched into the foil at most 25 holes with a minimum diameter of 0.5 mm. The result is that a well-defined reading off of the colour can no longer be guaranteed. On the other hand, fine perforations in punched foils tend to close up again because of the cold flow of the synthetic resin; furthermore, perforations give rise to

unevennesses of the foil and thus result in disturbing reflections of the surface.

A further attempt to solve the problems which occur resulted in the provision of a comparatively large intermediate space between the test paper and the synthetic resin foil (cf. published German Patent Specification No. 1,940,964). However, this results in increased bleeding effects, especially in the case of comparatively long immersion or in the case of investigations in flowing liquids, especially in a stream of urine; furthermore, the production of the test strips according to published German Patent Specification No. 1,940,964 is relatively complicated and thus expensive.

Surprisingly, we have now succeeded in producing test strips which, in comparison with those previously known, represent a significant advance since they do not suffer from the known disadvantages and, in addition, possess a number of additional advantages.

We have now found that especially storage-stable, non-bleeding indicator test strips, which are protected from contact and are free from delay, for the detection of substances in solution in liquids, especially in body fluids, are obtained when at least one carrier containing reagents and connected to a substrate serving as a holder, is covered with a light-permeable meshwork of thin filaments.

Consequently, according to the present invention, there is provided a test strip for the detection of substances in solution in liquids, especially in body fluids, comprising a substrate serving as a holder to which is attached at least one reagent carrier member containing reagents and having opposed surfaces, one surface of the carrier member or of each carrier member being adjacent to the holder and the other surface thereof, which is not adjacent to the holder, being provided with a covering, the covering consisting of a light-permeable meshwork of thin filaments, i.e. a meshwork which is transparent or translucent.

Surprisingly, the fine meshwork protects the underlying reagent-containing carrier member against manual contact and against external influences, although the holes in the meshwork can amount to 50% and more of the total surface area of the meshwork. Since the liquid to be investigated penetrates preponderantly through the holes of the meshwork, disturbing chromatographic effects can no longer occur. For this reason, it is now not also possible but, indeed, advantageous to apply the meshwork very closely to the carrier member.

It was to have been expected that the use of a meshwork as a covering for the reagent-containing carrier member would, because of insufficient covering, lead to a no longer acceptable increase of the bleeding effect, especially in the case of using the test strip in a stream of urine. However, we have, surprisingly, found that the opposite is the case and that the meshwork acts strongly counter to this type of disturbance to such an extent that the test strips according to the present invention, even in the case of very considerable wetting, such as by prolonged immersion in urine or by wetting in a stream of urine, are not washed out. It is also surprising that the reading off of even very slight colour changes is in no way impaired by the meshwork; on the contrary, the meshwork brings about an equalisation of the colour shade produced, which is pleasant to the eye and readily evaluable, the structure of the meshwork thereby not appearing. A further advantage of the test strips according to the present invention is the immeasurably rapid wetting of the reagent-containing carrier which gives a delay-free, readable colour reaction. Thus, the test strips according to the present invention can provide, for the first time, reproducible analytic values which are independent of the time of immersion. This signifies a considerable increase in the degree of certainty in the use of the test strips.

For a better understanding of the present invention, several embodiments thereof will now be described in more detail, with reference to the accompanying drawings, in which:

Fig. 1 is an enlarged cross-section of the lower part of a test strip according to the present invention;

Fig. 2 is an enlarged front view of the lower part of a test strip according to the present invention;

Figs. 3 and 5 show test strips according to the present invention, each having one reagent-containing carrier member, and

Figs. 4 and 6 show test strips according to the present invention, each having two reagent-containing carrier members.

In Figs. 1-6 of the accompanying drawings, on to a holder 2, which preferably consists of a stiff or rigid synthetic resin foil, there is applied an adhesive layer 3, which completely or partially covers the surface of the holder 2. At least one reagent-containing carrier member 4 is provided on the lower end of the holder 2, one surface of the carrier member 4 being either in direct contact with the holder 2 or with the adhesive layer 3 or with an intermediate layer of absorbent material.

A meshwork 1, which is of larger dimensions than the carrier member 4, covers the surface area 6 of the carrier member 4 which is not facing the holder, peripheral zones 8 of the meshwork 1 projecting beyond the carrier member 4 and being firmly connected, by means of the adhesive layer

3, with the holder 2. The meshwork 1 preferably lies directly upon the carrier member 4 so that the intermediate space between the meshwork 1 and the carrier member 4 is kept as small as possible.

The holder 2 is preferably made from a synthetic resin, for example, polystyrene, polyvinyl chloride, a polyester or a polyamide. However, it is also possible to impregnate an absorbent material, for example wood, paper or cardboard, with a water-repellent material or to cover an absorbent material with a water-resistant film. As hydrophobing agents, there can thereby be used, for example, silicones or hard fats, and as film-forming agents, there can be used, for example, nitrocellulose or cellulose acetate. Other examples of materials which can be used for the holder include metal foils and glass. The holder materials can be colourless and transparent but advantageously there are used opaque materials which, for increasing the colour contrast, can also be appropriately coloured.

The adhesive layer consists, for example, of a hot-sealable material, for example, polyethylene, a fusion adhesive or a cold hardenable adhesive. There can, indeed, be used any layer which securely bonds the meshwork 1, in the production of the test strips, to the holder 2 when, after the production, it can be completely hardened thermally, chemically or by drying processes with sufficient speed. The hardening of the adhesive layer 3 is necessary because the peripheral zones 8 of the meshwork 1 are, in many cases, completely embedded in the adhesive layer 3. In such cases, the adhesive layer passes through the holes in the meshwork and covers the meshwork 1 on the upper side thereof.

The carrier member preferably consists of an absorbent material, for example, filter paper or synthetic resin fleece, impregnated with test reagents and possibly with adjuvants, for example buffers or wetting agents. The carier member is normally applied to films of holder material in the form of bands. However, it is also possible to mix the detection reagents with neutral solid materials and optionally with a binding agent and a solvent and to coat the holder material with the paste so obtained, followed by drying. As neutral solid materials, there can be used, for example, cellulose or gypsum. Furthermore, the carrier member can consist of a hydrolysis resistant film as described and claimed in our British Patent Specification No. 1.159,627.

The meshwork 1 can consist of regularly woven filaments in the form of a fabric with weft and warp threads or can be in the form of a unwoven fabric. It is also possible to use thin felt- or fleece-like meshworks 1, in which the fibre structure is not uniform, provided that they have the necessary light-permeability and stability. It is preferred to use synthetic resin fabrics of monofile or spun filaments which can consist of cellulosic materials, for example cotton, cellulose, flax or sisal, proteinaceous materials, for example, wools or silk, or synthetic resins, for example polyamides, polyesters, polyethylene, polypropylene, polyvinyl chloride or polyacrylonitrile, or of a large variety of co-polymers. In some cases, it is also possible to use fine metallic fabrics. The diameter of the fibres used is expediently 5-200μ and preferably 20-100μ, the free surface left by the holes expediently being 30-80%, preferably 40-60%, of the total surface area. Within the given limits, the meshwork can be varied, depending upon the colour reaction of the reagent-containing carrier member. Normally, there are used meshworks of colourless material. However, with coloured meshworks, there are obtained mixed colours with the colours of the reagent-containing carrier member, which can sometimes increase the contrast. In addition, it is also possible to impregnate the meshwork with reagents which only penetrate into the reagent-containing carrier member upon wetting. This separate impregnation is recommended when there is a possibility that two or more detection reagents and/or adjuvants might react together during storage.

The connection of the meshwork 1 with the adhesive layer 3 can, depending upon the nature of the material used, be carried out by the application of pressure and/or by heating or by high frequency or ultrasonically. If the holder 2 consists of a softenable synthetic resin, for example polyvinyl chloride, then this can itself serve as the adhesive layer 3. The connection with the meshwork 1 then takes place by direct welding or by pressure after swelling the surface with a suitable solvent, for example with methylene chloride. The meshwork can, however, also be connected, for example, by partial sealing only with the carrier member 4.

The test strips according to the present invention are preferably produced in the following manner: broad bands of holder material provided with the adhesive layer 3, together with narrow bands of reagent paper, which is used as carrier member 4, and somewhat broader bands of the meshwork 1, are thermally sealed together along the projecting surfaces 8, whereafter the resulting test strip band is cut transversely into test strips of the desired width.

Depending upon whether it is desired to produce mono- or multi-test strips, one or more reagent-containing carrier members 4 can be applied in parallel bands to the

holder foil 2. Figs. 4 and 6 of the accompanying drawings illustrate embodiments of twofold test strips. The cut surfaces 7 of the carrier member 4 resulting from the cutting of the above-mentioned test strip bands need not be covered with the meshwork 1 because the free edge surface 7 is relatively small.

In a particular embodiment of the test strips according to the present invention (cf. Fig. 5 of the accompanying drawings), below the carrier member 4, i.e. between the carrier member 4 and the holder 2 or adhesive layer 3, is placed an absorbent material 9, which does not contain any reagents. In some cases, this increases the sensitivity of the carrier member and protects it against possible adverse effects of the adhesive layer.

In Figs. 3 and 4 of the accompanying drawings, the adhesive layer 3 is supplied to the holder 2 in the form of narrow strips. In this way, there is avoided any undesired contact between the reagents with substances in the adhesive layer. If the carrier foil is covered completely or preponderantly with the adhesive layer 3, this can be used additionally to fix to the carrier 2 a covering foil 5 (cf. Fig. 5 of the accompanying drawings) provided, for example, with instructions or comparative colours.

The test strips according to the present invention are particularly useful for the investigation of body fluids, especially of urine; however, in the case of appropriate modification of the detection reagents, they can also be of quite general applicability. It is obvious that turbid liquids, such as blood or urine with a high content of sediment, must possibly be centrifuged or filtered before carrying out the investigations. Since the test strips according to the present invention are very quickly and easily wetted, it is even possible to analyse, without any time delay, viscous liquids, for example serum or secretions from or on mucous membranes, for example saliva.

The following Examples are given for the purpose of illustrating the present invention:—

Example 1.
pH test strips

Filter paper (Schleicher & Schüll No. 2316) is impregnated with the following solution:

methyl red	0.08 g.
bromothymol blue	1.00 g.
methanol	ad 1000 ml.

dried in a current of warm air and cut into strips with a width of 6 mm.

These bands are sealed, by means of hot rollers, between a 60 mm. wide band of melt wax-coated polyester foil and a 12 mm. wide band of polyester fleece (15 g./m^2) in such a manner that the middle of the test paper comes to lie 6 mm. from the lower edge of the polyester band and below the middle of the fleece band. The hot rollers used are provided with recesses corresponding to the position of the test paper. If, under the pH test paper, there is laid a conventional filter paper of the same width, then the recesses on the hot rollers can be omitted.

The finished sealed band is then cut transversely into strips of 6 mm. breadth.

When these test strips are dipped into solutions of pH 5-8, then, depending upon the pH, there are obtained colorations from yellow to blue which extend uniformly over the whole of the test area.

When the pH test paper is sealed in conventional manner between polyethylene-coated polyester foils then, due to chromatographic effects and air bubbles, under unfavourable conditions, disturbances can occur.

Example 2.
Protein test strips

Filter paper (Schleicher & Schüll No. 2316) is successively impregnated with the following solutions:

I	sodium citrate	130.6 g.
	citric acid	46.6 g.
	lauroyl sarcosine	0.8 g.
	water	500.0 g.
	methanol	ad 1000 ml.
II	magnesium sulphate	59.4 g.
	tetrabromophenolphthalein ethyl ester	0.5 g.
	methanol	ad 1000 ml.

dried and cut up into 6 mm. wide strips.

The test paper is sealed between polyester foil and fleece and cut transversely in the manner described in Example 1.

When the test strips thus obtained are dipped into protein-containing urine, then uniformly green to blue colorations of the test zone are obtained. When the test strips are held for 5 seconds in a stream of urine, then the same colour is obtained as in the case of being dipped into the same urine. In particular, protein-free urine gives a negative reaction in both cases, this being indicated by a pale yellow colour. Conventional sealed in or sealed on test strips can, in contradistinction thereto, indicate a faintly positive reaction due to washing out effects.

Example 3.
Combined protein and pH test strips.

pH and protein test papers are produced in the manner described in Examples 1 and 2 and sealed in in such a manner that they come to lie on the same test strip next to one another at a distance of about 3 mm.

When these test strips are dipped into appropriate test solutions, then the same colours are obtained as with the corresponding single test strips. These colorations also do not change over a comparatively long period of time.

When, however, the test papers are only sealed on next to one another, then, shortly after dipping into the test solution, the side of the protein test zone nearest to the pH test zone becomes more deeply green coloured, whereas the corresponding side of the pH test zone takes on a colour corresponding to a more acidic pH value. These results are due to a diffusion of the acidic buffer from the protein test zone to the pH test zone through the connecting film of liquid.

Example 4.
Urobilinogen test strips.

Filter paper (Schleicher & Schüll No. 2312) is impregnated with a solution of the following composition:

4-cyclohexylaminobenzaldehyde	1.0 g.
oxalic acid	200 g.
methanol	ad 1000 ml.

dried and cut up into 6 mm. wide bands.

The test paper band is, in the manner described in Example 1, sealed in between bands of polyethylene-coated polyester foil and nylon fabric (60μ filament thickness, 45% free hole surface) and cut transversely.

When these test strips are dipped into urobilinogen-containing urine, then a completely uniform red coloration of the test zone is formed which permits a reproducible, semi-quantitative determination of the urobilinogen.

When the test paper is sealed between two polyethylene-coated polyester foils and only dipped briefly into the urine, then red strips are only obtained on the open edges of the test zone, whereas the middle remains white.

Example 5.
Glucose test strips.

Filter paper (Schleicher & Schüll No. 23 SL) is impregnated with the following impregnation solution:

o-tolidine	4 g.
peroxidase	0.12 g.
glucose oxidase	13.0 g.
tartrazine	0.9 g.
ethanol (44%)	ad 1000 ml.

dried and cut up into 6 mm. wide bands.

On to a polyvinyl chloride band of 60 mm. breadth are applied, from two nozzles, on the lower edge and at a distance of 10 mm. therefrom, 3 mm. strips of an air-hardenable cyanoacrylate adhesive. Shortly thereafter, the test paper is laid between the strips and a 12 mm. wide band of polyester fabric (30μ filament thickness and 45% free hole surface) stuck thereover. After hardening of the adhesive, the whole band is cut up transversely into strips of 6 mm. breadth.

When these test strips are dipped into glucose-containing solutions, for example into urine, then, depending upon the glucose content, there is obtained a uniform, more or less green coloration of the test zone.

When test strips are sealed, in conventional manner, between two polyethylene-coated polyester foils and then dipped into urine, then there is formed an air bubble with a dark green colour fleck which is brought about by the stronger atmospheric oxidation of this area.

Example 6.
Hydrogen peroxide test strips.

A mixture of the following composition:—

polyvinyl propionate dispersion	45.0 g.
phosphate buffer 0.4M (pH 5.5)	45.0 nil
sodium alginate	0.5 g.
sodium lauryl sulphate	0.6 g.
o-tolidine	0.2 g.
peroxidase	0.02 g.
methanol	6.0 g.

is coated, with a layer thickness of 350μ, on to a polyvinylidene chloride-coated paper and dried.

6 mm. wide bands of this paper provided with the reagent film are then further worked up, as described in Example 5, to give test strips but using a nylon fabric (60μ filament thickness and 45% free hole surface) instead of a polyester fabric.

When test strips of this type are dipped into hydrogen peroxide-containing solutions, then a uniformly blue coloration is obtained, the depth of which depends upon the hydrogen peroxide concentration.

When, on the other hand, the paper provided with the reagent film is briefly dipped into the liquid and then removed immediately, there is obtained a non-uniform reaction, since the liquid is not uniformly distributed on the surface of the film.

Example 7.
Nitrate test strips.

Filter paper (Schleicher & Schüll No. 2316) is impregnated with a solution of the following composition:—

sulphanilamide	2.0 g.
α-naphthylamine	1.2 g.
tartaric acid	25.0 g.
methanol	ad 1000 ml.

dried and cut up into 6 mm. wide bands.

The test paper is, in a manner analogous to that described in Example 1, sealed between a melt wax-coated polyester foil and a nylon fabric (see Example 6) and

cut up into 6 mm. broad strips.

After dipping into nitrite-containing test liquids, the test strips show mor or less red colorations. They ar especially readily usable in strongly viscous solutions, such as occur in the production of sugar. Nitrile test papers sealed between polyethylene-coated polyester foils cannot be used for this purpose.

WHAT WE CLAIM IS:—

1. Test strip for the detection of substances in solution in liquids, comprising a substrate serving as a holder to which is attached at least one reagent carrier member containing reagents and having opposed surfaces, one surface of said carrier member or of each said carrier member being adjacent to the holder and the other surface thereof, which is not adjacent to the holder, being provided with a covering, said covering consisting of a light-permeable meshwork of their filaments.

2. Test strip according to claim 1, wherein the covering consists of a synthetic resin fabric with a filament diameter of 5-200μ.

3. Test strip according to claim 2, wherein the covering consists of synthetic resin fabric with a filament diameter of 20-100μ.

4. Test strip according to any of the preceding claims, wherein the free surface area left by the holes in the covering is 30-80% of the total surface area.

5. Test strip according to claim 4, wherein the free surface area left by the holes in the covering is 40-60% of the total surface area.

6. Test strip according to any of the preceding claims, wherein the covering consists of a fleece or or a woven fabric.

7. Test strip according to any of th preceding claims, wherein the covering has peripheral zones which project beyond the carrier member and are connected partially or wholly with the holder by means of an adhesive.

8. Test strip according to any of the preceding claims, wherein the carrier member consists of an absorbent paper or fleece.

9. Test strip according to any of the preceding claims, wherein the holder is made from a synthetic resin or from an absorbent material impregnated or coated with a water-repellent material.

10. Test strip according to claim 1 for the detection of substances in solution in liquids, substantially as hereinbefore described and exemplified and with reference to any of the Figures of the accompanying drawings.

11. Process for the production of test strips according to claim 1, substantially as hereinbefore described and exemplified.

12. Test strips, whenever produced by the process according to claim 11.

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1349623 COMPLETE SPECIFICATION

1 SHEET *This drawing is a reproduction of the Original on a reduced scale*

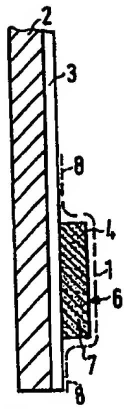

FIG. 1

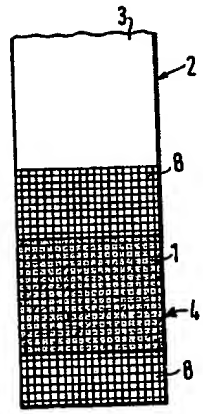

FIG. 2

FIG. 3

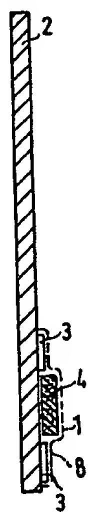

FIG. 4

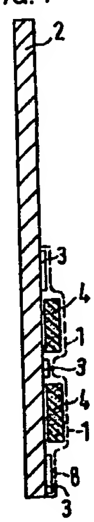

FIG. 5

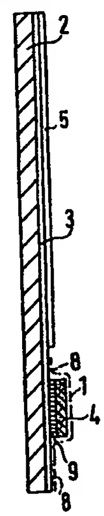

FIG. 6